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Experience with opioids does not modify the brain network involved in expectations of placebo analgesia

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Abstract

Placebo analgesia (PA) is defined as a psychobiological phenomenon triggered by the information surrounding an analgesic drug instead of its inherent pharmacological properties. PA is hypothesized to be formed through either verbal suggestions or conditioning. The present study aims at disentangling the neural correlates of expectations effects with or without conditioning through prior experience using the model of PA.

We addressed this question by recruiting two groups of individuals holding comparable verbally-induced expectations regarding morphine analgesia but either (i) with or (ii) without prior experience with opioids. We then contrasted the two groups' neurocognitive response to acute heat-pain induction following the injection of sham morphine using electroencephalography (EEG). Topographic ERP analyses of the N2 and P2 pain evoked potential components allowed to test the hypothesis that PA involves distinct neural networks when induced by expectations with or without prior experience.

First, we confirmed that the two groups showed corresponding expectations of morphine analgesia (Hedges' $g_s < .4$ positive control criteria, $g_s = .37$ observed difference), and that our intervention induced a medium-sized PA (Hedges' $g_{av} \geq .5$ positive control, $g_{av} = .6$ observed PA). We then tested our hypothesis on the recruitment of different PA-associated brain networks in individuals with versus without prior experience with opioids and found no evidence for a

List of abbreviations: ASR, Artifact Subspace Reconstruction; CHEP(S), Contact Heat Evoked Potential (Stimulator); CMS-DRL, Common Mode Sense-Driven Right Leg; CondExp, Conditioned Expectations (group); DBP, diastolic blood pressure; EEG, electroencephalography; ERP, event-related potential; GFP, Global Field Power; GHQ, General Health Questionnaire; GMD, Global Map Dissimilarity; HR, heart rate; IPA, in-principle-acceptance; LEP, laser-evoked potential; LPP, late positive potential (component); MAD, median absolute deviation; MAEQ, Morphine Adverse Effects Questionnaire; MAExpQ, Morphine Analgesia Expectations Questionnaire; MFS, Medical Fear Survey; PA, placebo analgesia; PBO, placebo; POI, period of interest; PostInject, post-injection (phase); PreInject, pre-injection (phase); SBP, systolic blood pressure; SESOI, smallest effect size of interest; TF, time frame; UncondExp, Unconditioned Expectations (group); VAS, Visual Analogue Scale.

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topographic N2 and P2 ERP components difference between the two groups. Our results thus suggest that in the presence of verbally-induced expectations, modifications in the PA-associated brain activity by conditioning are either absent or very small.

KEYWORDS
EEG, ERP topography, expectations, pain, placebo

1 | INTRODUCTION

Placebo analgesia (PA; *see for comparable acronym use* Koban et al., 2012; Nakamura et al., 2012; De Pascalis & Scacchia, 2019) is a psychobiological reaction elicited by the contextual information associated with a non-active drug (i.e., placebo drug) rather than by any inherent active pharmacological properties. As for other placebo effects, PA is thought to result from expectations formed through verbal suggestions (unconditioned expectations) and conditioning (conditioned expectations). Yet, while verbal suggestions and conditioning induce corresponding behavioural placebo effects, indirect evidence suggest that they might involve different brain networks. The present study aims at confirming this hypothesis to deepen the fundamental understanding of the placebo effects.

Support for the assumption that distinct brain networks are involved in conditioned and unconditioned expectations notably comes from electrophysiological data. Colloca et al. (2009), for instance, showed that while PA induced by both conditioned and unconditioned expectations is associated with reduced N2-P2 event-related potential (ERP) complex amplitude, this effect is larger with conditioning. This pattern suggests a stronger involvement of early nociceptive brain mechanisms in conditioned than unconditioned expectations. In line with this finding, Carlino et al. (2015) report that PA -related reductions in N2-P2 amplitude occur when conditioning is associated with verbal information, but not when conditioning is engaged alone. Finally, psychopharmacological studies show that while conditioned PA depends on the drug's pharmacological effect, unconditioned PA mostly relies on the opioid system, further suggesting that different neurochemical pathways support each type of PA (*see for a review* Okusogu & Colloca, 2019; and for other investigation on this question: Amanzio & Benedetti, 1999; Bartels et al., 2014; Benedetti et al., 2003; Colloca et al., 2008; Colloca & Benedetti, 2009; De Jong et al., 1996; Montgomery & Kirsch, 1997; Reicherts et al., 2016; Voudouris et al., 1990).

Additionally, striatal regions of the reward system contribute to pre-cognitive anticipation of PA in conditioning (*see for reviews* de la Fuente-Fernández, 2009; Peciña et al., 2014). Since conditioning is by definition implicit (Bąbel, 2019; Benedetti et al., 2003), only implicit mechanisms would recruit this system. Hence, these evidence further suggest that different brain networks are involved in conditioned and unconditioned expectations.

We tested this hypothesis by focusing on the N2 and P2 ERP components during heat-evoked pain responses; The N2 and P2 components arise respectively 200–400 and 300–500 ms after the stimulation of Aδ fibers (Bromm & Treede, 1987) and originates from brain areas also involved in PA (Apkarian et al., 2005; *see for reviews* Benedetti, 2014; Wager & Atlas, 2015). These early components are sensitive to nociceptive stimulation (Wager et al., 2006) and are associated with the magnitude of pain perception (Colloca et al., 2009; Garcí-Larrea et al., 1997; Iannetti et al., 2005; *for reviews see* Colloca, 2014; Legrain et al., 2002). At the cognitive level, the N2 component is hypothesized to index top-down attentional mechanisms, while the P2 is more sensitive to bottom-up attentional orienting mechanisms as those modulated by the probability of stimulus occurrence (Legrain et al., 2002; Legrain et al., 2003; Legrain et al., 2005). We chose to focus on these components to compare the placebo effects induced by two types of expectations because even if they are not specific to PA, they are minimally involved in it (Carlino et al., 2015; Colloca et al., 2009). Current accounts for the neural bases of placebo effects indeed advance that placebos mimic the neurobiological action of the substituted drugs (*see for reviews* Benedetti, 2014; Benedetti et al., 2011; Colloca, 2014; Haour, 2005; Kirsch, 1997; Meissner et al., 2011; Pacheco-López et al., 2006; Stewart-Williams & Podd, 2004; Wager & Atlas, 2015). We thus expect placebo morphine to modulate the same brain markers that would be affected by real morphine, and in turn the N2 and P2 components, two reliable indexes of antinociceptive drugs-induced analgesia (Truini et al., 2010).

The literature reviewed, however, relies on methods with limited neurophysiological interpretability; they could notably not differentiate between purely

quantitative from qualitative modulation of brain activity. In addition, they focused on laboratory-induced non-pharmacological conditioning. They could thus not provide direct evidence that the brain networks mediating pre-established pharmacological conditioning effects differ from those involved in verbally-induced expectations.

To fill these gaps, we compared the neurocognitive responses to acute pain induction after the stimulation of Aδ fibers and after sham morphine administration between groups having the same verbally-induced expectations on the effects of morphine, but either with versus without an actual experience of opioids. This contrast enabled us to address our hypothesis because only the group with experience of opioids had formed conditioned expectations through prior experience. Specific questionnaires and inclusion criteria were used to control that morphine-related verbal suggestions were homogenous within- and between-group.

We identified whether each type of expectation engages distinct brain networks by comparing the spatial distribution of the N2-P2 scalp field potentials between the conditioned versus unconditioned expectations groups. We statistically compared the N2-P2 components topography with the so-called Global Map Dissimilarity (GMD), an index computed as the root mean square of the difference between the potentials measured at each electrode. This approach allowed to test our hypothesis because differences in ERP topography necessarily follow from alterations in the configuration of the underlying brain generators (Murray et al., 2008; Tzovara et al., 2012). Hence, if we observed different N2 and/or P2 ERP topographic maps (i.e., GMD differences) between the conditioned versus unconditioned group, our hypothesis that the two types of expectations involve different brain networks would be confirmed. Whether the topographic modulation manifest during the N2 and/or P2 component further provided information on whether each type of expectations differ at the level of the network involved in top-down or bottom-up attention to noxious stimulations.

2 | METHODS

2.1 | Participants

Participants were recruited with public advertisement.

Inclusion criteria include: Male or female, right-handed, 18 to 50 years old, French speakers, and a regular consumption of less than or equal to 21 units of alcohol/week to exclude individuals with a pathological relationship to alcohol.

To improve the homogeneity of the expectations in both groups, we controlled that they had neutral to positive prior experience with opioids (no severe adverse effects or intolerance), and thus that they had formed neutral to positive expectations regarding morphine analgesic properties (i.e., score > or = to 18 at the Morphine Analgesia Expectations Questionnaire, MAExpQ; *see* Section 3.5).

For the conditioned group, we controlled that they had comparable levels of PA conditioned expectations by ensuring that they had been treated with opioids as part of a medical treatment for a 1- to 6-month continuous duration per treatment course in the last 3 years maximum (several courses allowed during this period, provided there was a >1-month free time interval between them). Additionally, the pain-causing disease should not have been a cancer (unless completely healed) or a neurological disease (i.e., neuropathic pain). Yet, since pure conditioning (i.e., without verbal suggestions) is by nature implicit (*see* Bąbel, 2019) variation at this level may only have had a limited influence over our results, if any.

Exclusion criteria include: Needle phobia (score >8 on the Injections and Blood Draws subscale of the Medical Fear Survey, MFS; *see* Olatunji et al., 2012), history of substance-related addictive or misuse disorders (alcohol or other drugs), history of diagnosed neurological or psychiatric disorders (including acute and chronic pain syndromes), skin affliction of the forearms (*see* Schenk et al., 2014).

All eligible participants provided informed consent before beginning the experiment. Participants were allowed to withdraw their participation anytime without needing to provide explanations. We compensated participation to the study (at a rate of 25 CHF/hour; including transportation costs) even in case of withdrawal.

2.2 | Hypotheses and power analyses

We hypothesized that participants with prior experience with opioids (i.e., CondExp group) would recruit different neural networks to generate PA compared to individuals without prior experience with opioids (i.e., UncondExp group). This difference was expected to manifest during the N2 and/or P2 ERP components time windows, following the onset of heat-pain stimulations. Namely, if the effect manifested during the N2 time window we would have concluded that conditioning in PA influences attention towards noxious stimuli (i.e., top-down attentional mechanisms) while if it manifested for P2 we would have assumed that conditioning affects the way noxious

stimuli attracts attention (i.e., bottom-up attentional mechanisms; *see* Legrain et al., 2005, Legrain et al., 2003, Legrain et al., 2002).

2.2.1 | Power analysis

The sample was determined by computing a Monte Carlo power analysis (Muthén & Muthén, 2002; Paxton et al., 2001) on the contrast used to test our main hypothesis with a custom R script (R Core Team, 2020) relying on the 'Superpower' R package (Caldwell & Lakens, 2019).

For that purpose, we proceeded as follows:

1. First, we estimated the smallest absolute effect size of interest (SESOI) for our GMD contrast. Since GMD is a single value index of the differences in topography between two ERP fields (Brunet et al., 2011), the GMD group mean and standard deviation cannot be computed. We thus relied on the maximal single-electrode voltage difference across the whole montage at a given time frame, an index highly correlated with the GMD (correlation of $r = .79$ and $r = .81$ during the period of the N2 component in two independent datasets) (Najberg et al., 2020; Ribordy Lambert et al., 2020). On this basis, we identified the SESOI as 2.99 mV based on those observed in previous contrasts close to the present study in terms of the expected variation in network configuration (Colloca et al., 2009; Ribordy Lambert et al., 2020). We conservatively decided to take only half of the absolute effect size reported in Colloca et al. (2009) since this study included a small sample size (i.e., $N = 16$ per group) and may thus have detected an inflated effect size. Consequently, we restricted our SESOI as half of the 2.99 mV: 1.45 mV. Considering the adjusted SESOI and the SD of each group, the smallest relative effect size corresponded to a Hedge's $g_s = .56$.
2. We then calculated the between-subject variance of the voltage amplitude difference during the N2 component in Ribordy Lambert et al. (2020): 2.6 mV.
3. Based on the SESOI and variance parameters identified in steps 1 and 2, we generated 10,000 simulated datasets per conditions (conditioned and unconditioned groups) for total sample sizes ranging from 50 to 80 participants by steps of 2. For each simulation, we randomly drew data from the generated normal distributions and computed the power of each sample size to detect our effect of interest by extracting the percentage of p values that fell below our target alpha threshold of .05.
4. Finally, we identified the minimal sample required to detect our smallest effect size of interest with a .9 power.

The R script of the simulation can be found on Zenodo (https://doi.org/10.5281/zenodo.6043795).

To detect a moderate effect size of Hedge's $g_s = .56$ with a power of .9 and an alpha threshold of .05, a total sample of $N = 66$ (i.e., $n = 33$ per group) was considered (*see* Figure 1). Drop-outs and other excluded participants were replaced to maintain this sample size in the final analyses.

2.2.2 | Experimental design

We tested our hypothesis using the analgesic effects of placebo morphine after acute pain induction (i.e., PA). The target population was manageable to recruit because knowledge of morphine antalgic properties is largely shared (De Sola et al., 2018), with ~8% of individuals having actually experienced its effects (Wertli et al., 2017 for data in Swiss populations).

We used a two-cell between-subjects design: (i) Conditioned expectations group (CondExp): Individuals holding neutral or positive expectations of morphine analgesia and with prior positive experience with opioids, namely they had been treated with opioids as part of a

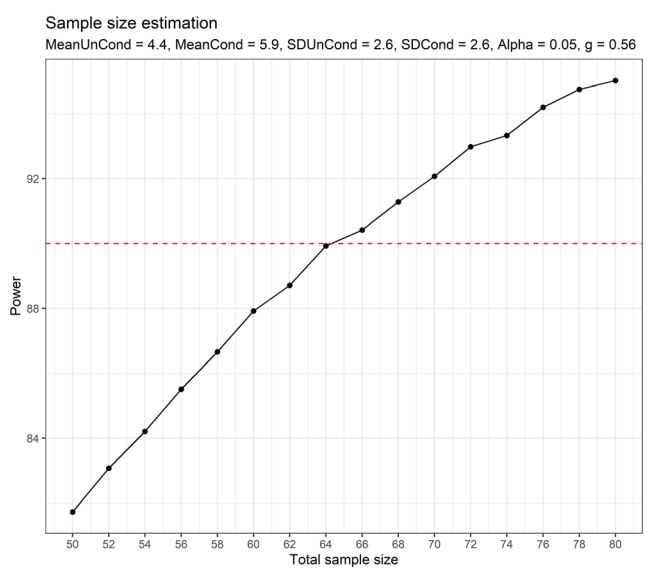


FIGURE 1 Representation of the power that can be reached according to each sample size, from $N = 50$ to $N = 80$ by steps of 2, based on data from Colloca et al., 2009 Hence, to reach a 90% power, a sample of $N = 66$ for both groups was required. MeanUnCond/SDUnCond = Mean/SD of UncondExp group; MeanCond/SDCond = Mean/SD of CondExp group; Alpha = Alpha threshold; g = Hedge's g_s

medical treatments according to criteria detailed in Section 3.1; and (ii) Unconditioned expectations group (UncondExp): Individuals holding neutral to positive expectations of morphine analgesia but without prior experience with opioids.

Due to the lack of a conditioning-only group, our design could not rule out the presence of a conditioning-by-expectations interaction effect in the CondExp versus UncondExp contrast. Hence, interpretations of our results solely went in the direction that adding conditioning with prior experience to expectations would recruit different neural networks than expectations alone to engender PA.

We were aware that our design remained less well-controlled than laboratory-based conditioning designs with administration of precise molecule, dose and number of runs (*see*, *for an example*, Amanzio & Benedetti, 1999). Nevertheless, our approach had the advantage of providing stronger ecological relevance while replicating earlier laboratory-based findings in an observational paradigm and extending them at the electrophysiological level.

2.3 | Procedure

The experimental session lasted around 1.75 h. The procedure of the session is summarized in Figure 2; it consisted of the following steps:

As part of a two-stage informed consent procedure, participants gave their initial written consent for the study before data collection (i.e., only partial information disclosure) while a second consent was provided as soon as the complete information was revealed at the end of the session. In the first informed consent document, participants' beliefs in the analgesic properties of Morphine were reinforced with the following paragraph: 'Opium, from which morphine is extracted, was cultivated and used by the Sumerians to treat pain already 5000 years ago. The extracted morphine was injected for the first time 150 years ago by the Irish surgeon Francis Rynd (1801-1861). It remains now the most widely used analgesic and is the standard against which new pain-relieving medicine are tested. From 33 to 100% of patients report that morphine can completely suppress their pain, depending on the route of administration (Walsh et al., 2006). Morphine is a very potent drug since, once injected, it reaches its peak concentration in the blood after roughly 15min and its analgesic effects can last up to 24hours'. Then, participants were screened regarding the inclusion and exclusion criteria (with the GHQ, MAExpQ and MFS questionnaires) and the electrodes for the electroencephalography (EEG) recording system were positioned on participants' head (duration ~15 min). Then, pain stimulation threshold was measured to determine the individual stimulation intensity that produces comparable pain feelings across participants (*see* Section 2.4.2).

Afterwards, participants' cardiovascular readings were assessed for the first time as part of the cover story (*see* Section 2.6.2) after which they underwent the pre-injection pain induction phase (i.e., PreInject phase) which lasted ~8 min. They were then administered the saline injection framed as containing morphine by licensed study personnel. They were instructed that the syringe contained a dose of 3.5-mg morphine (*see for a review* Sverrisdóttir et al., 2015) corresponding to half the dose that was routinely administered subcutaneously in a

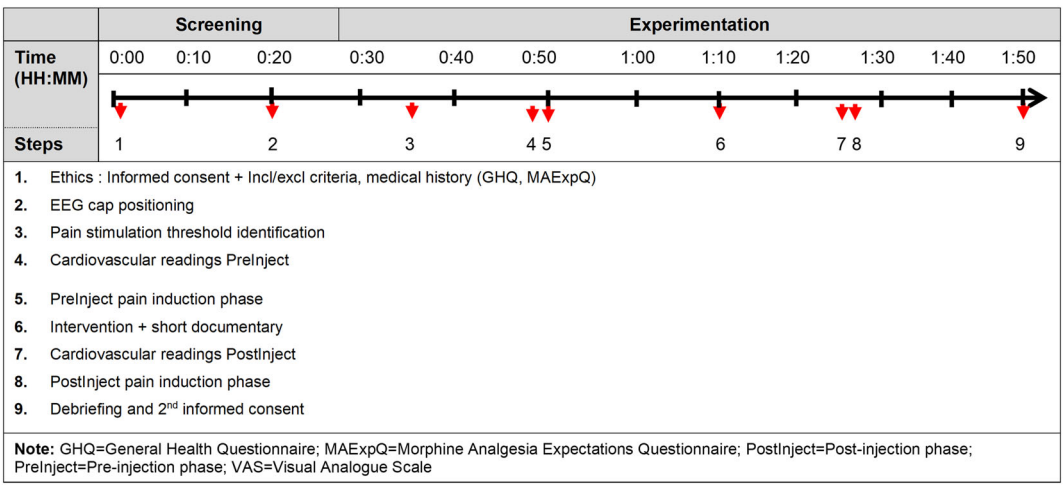


FIGURE 2 Summary of the procedure. GHQ = General Health Questionnaire; MAExpQ = Morphine Analgesia Expectations Questionnaire; PostInject = post-injection phase; PreInject = pre-injection phase; VAS = Visual Analogue Scale

hospital setting after a surgery or an injury, or to manage cancer pain or pain after a heart attack (National Health Institute, 2018). Fifteen minutes after the injection, participants had their cardiovascular function assessed for a second time again as part of the cover story (see Section 2.6.2) and then they underwent the post-injection pain induction phase (i.e., PostInject phase), again lasting ~8 min. Finally, participants received a debriefing information which disclosed the deception involved in the study and enabled them to react to it. They further provided a second informed consent, necessary to ensure that they maintained their consent after having received all information on the study.

2.4 | Task

2.4.1 | Pain stimulation device

Painful stimulations were performed with the PC-controlled PATHWAY CHEPS device (Medoc Advanced Medical Systems, Rimat Yishai, Israel). The thermofoil thermode had a surface of 27 mm in diameter (527.5 mm^2). The thermode was placed on the left volar forearm and held by the experimenter while the stimulation site was moved between one of three positions 5 cm apart[1] within the same dermatome after each trial to avoid sensitization and/or habituation after repeated pain stimuli (*for a similar procedure, see* Warbrick et al., 2009). The thermofoil temperature ranged between 32°C and 52°C with a heating rate of 70°C/s and was further cooled down with a rate of 40°C/sec (*for a similar procedure, see* Aslaksen et al., 2011).

2.4.2 | Identification of pain stimulation threshold

The intensity of the experimental stimulation trials was defined at the individual-subject level according to Schenk et al. (2014). In a first step, we relied on the methods of limits to assess the heat pain threshold and tolerance with temperature increasing at a rate of .3°C/s (Fruhstorfer et al., 1976). We applied two steadily increasing thermal stimulations until participants indicated orally when (i) they started to experience pain (i.e., transition from warm sensation to pain feeling) and (ii) when the pain was unbearable (i.e., pain tolerance). In a second step, 11 pain stimulations were applied to reach an individualized temperature eliciting a pain level of 8 or higher on the Visual Analogue Scale (VAS; 0–10) or 52°C (if the corresponding VAS rating was lower than 8). The stimulation temperatures ranged from 42°C to 52°C and the temperature increased by steps of 1°C after each trial. Correspondingly, the temperature equivalent to a level of pain of 8 or higher on the VAS scale[2] was then used to induce pain throughout the session.

2.4.3 | Experimental painful stimulation

The experimental painful stimulation paradigm was divided into two phases of two blocks of 12[3] trials (i.e., 24 trials in total; *for a comparable procedure, see* Aslaksen et al., 2011). Each block was separated by a 5-min break. The first phase happened before the saline injection (i.e., PreInject phase) while the second phase happened 15 min after the saline injection (i.e., PostInject phase), which corresponded to the time necessary to reach the peak concentration with real morphine (Mazoit et al., 2007). The post-injection scores were further used for statistical comparisons of our main contrast of interest (see Section 2.2.2) while both pre- and post-injection scores were used for the sanity checks to ensure that placebo analgesia indeed occurred (see Section 2.4.5).

The CHEPS software enabled the randomization of the interval between two stimulations which was set between 10 and 15 s to minimize the predictability of pain onset (*see for a similiar procedure* Schenk et al., 2014). Each block of 12 trials thus lasted roughly 3 min (including the inter-stimulus intervals). Stimulus onset was indexed by a TTL-pulse issued from the CHEPS software to the EEG-amplifier as soon as the temperature starts increasing (*see for a similar procedure* Aslaksen et al., 2011).

2.4.4 | Pain perception ratings

After the 6th and the 12th trial of each block, participants had to rate the perceived intensity of the stimulation on a

[1]*The anticipated sensitization process after repeated painful stimulations at the same skin location was stronger than expected and the signal-to-noise ratio was improved by moving the thermode within the same dermatome after each trial. The editorial approval for the deviation was obtained on the 27 October 2021, after the commencement of data collection but prior to data analysis.*

[2]*We adjusted this criterion as we realized that a VAS = 6 corresponded to a peak temperature of 46–48°C and was thus too low to reach an optimal signal-to-noise ratio in the ERPs. Hence, we increase it to VAS = 8 to reach a temperature of ~51–52°C.*

[3]*Since we increased the target VAS from 6 to 8, we also reduced by half the number of trials to shorten the duration of painful sensations.The editorial approval for these two deviations was obtained on 27 October 2021, after the commencement of data collection but prior to data analysis.*

0–10 VAS scale, labelled respectively as 'no pain at all' to 'unbearable pain'. The VAS was displayed on a computer screen and subjects had to move a cursor to indicate the value corresponding to the intensity of their pain. Global scores were computed as the average of the painful ratings over the four trials of each phase.

2.4.5 | Sanity checks

The following data sanity checks were used to control that our two groups shared common verbally-induced expectations and that the manipulation induced PA, which were a prerequisite for our key CondExp versu UncondExp GMD differences of interest to be interpretable.

A. First, we ensured that participants from both groups shared common positive verbally-induced expectations of morphine analgesia to ensure that this factor did not confound our contrast of interest. To this aim, we controlled that the difference at the MAExpQ global score was smaller than Hedges' $g_s < .4$.
B. We then ensured that a placebo effect indeed occurred after the injection and thus that we could interpret any electrophysiological effect in terms of PA. To this aim, we used one-sided differences of Hedges' $g_{av} \geq .5$ (.9 power and alpha threshold of .05 for this contrast with our planned sample size) on the mean of VAS trials after the injection (PostInject phase) compared to trials before the injection (PreInject phase), on the whole population sample.

If the first sanity check (i.e., A) was not fulfilled, participants with the value farther from the overall pooled median were successively excluded and replaced until the groups were balanced (i.e., since all participants should share the same expectations).

If the second sanity check (i.e., B) was not fulfilled, participants not showing placebo effects with the highest value compared to the difference score between the Δ VAS PreInject − VAS PostInject phases[4] were successfully excluded and replaced until we reached the expected difference of Hedges' $g_{av} \geq .5$ between the phases.

[4]*The size of the placebo effect was initially calculated by comparing the individual's Post inject VAS to the group median VAS PostInject. Still, with this approach, individuals displaying a placebo effect but that have (by chance) a VAS PostInject close to the group VAS PostInject will be considered as not showing placebo effect. Thus, to efficiently exclude individuals experiencing no placebo effects, we instead computed a difference score between the Δ VAS PreInject – VAS PostInject.* (i.e. *emails exchange of the 19.11.2021).*

In the case where only one of the sanity checks was not fulfilled while the other one was, we will proceed to the exclusion and replacement of the participant.

2.5 | Questionnaires

- Demographics, measures of morphine analgesia expectations, as well as exclusion criteria were assessed with the following questionnaires:
- General Health Questionnaire (GHQ): custom-made 40-items questionnaire, self-assessment of overall health, sport activities, drug consumption habits and substance use and abuse (e.g., cigarettes and alcohol).
- Morphine Analgesia Expectations Questionnaire (MAExpQ): custom-made 9-items questionnaire, self-assessment of expectations regarding the analgesic properties of morphine and prior morphine or other opioids exposure.
- Morphine Adverse Effects Questionnaire (MAEQ): custom-made 12-items questionnaire, self-assessment of expectations of and prior experience with morphine or other opioids' side effects.
- Medical Fear Survey-Short Version (MFS): 25-items, self-assessment of medically related fears along five dimensions: Injections and Blood Draws, Sharp Objects, Blood, Mutilation and Examinations and Symptoms (*translated from* Olatunji et al., 2012). We exclusively focused on the Injections and Blood Draws subscale to exclude participants with needle phobia (*see* Section 3.1).

2.6 | Blinding procedures and cover story

2.6.1 | Saline injection

All subjects received a subcutaneous injection (i.e. better than intramuscular injection for morphine; Jin et al., 2015) of one bolus of saline (NaCl .9%) which was framed as containing real morphine, in the lower abdomen. The injection was performed by licensed study personnel.

2.6.2 | Cover story

We implemented a deceptive protocol (i.e., cover story) to maximize the credibility of our procedure as well as participants' expectations regarding the intervention. Previous evidence had demonstrated the usefulness of using cover stories when deceiving participants in placebo

manipulation experiments (see Elkins-Brown et al., 2018). Participants were instructed that the study aimed at clarifying the physiological mechanisms underlying the effects of morphine on acute pain in healthy populations. They were informed that, among others, we expected to observe a significant decrease in their cardiovascular readings roughly 15 min after morphine injection.

First, the injection was performed by licensed study personnel who were wearing the local hospital official coat and badge. The personnel followed the hospital safety guideline before beginning the injection by asking participants about their medical history, allergies, medication intake and assessed their cardiovascular function for the first time. We ensured that the cardiovascular readings that participants saw on the device's screen were not their own, but a measurement that was preprogrammed to display readings which were in the upper norm (e.g., heart rate (HR) = 95 beats/min; Systolic Blood Pressure (SBP) = 138 mmHg; diastolic blood pressure (DBP) = 74 mmHg; *taken from* Rouby et al., 1981).

Obviously since we injected saline, the licensed personnel let each participant know that his/her results to the safety assessments were fine and that he could proceed with the injection. Beforehand, he warned the participant regarding the most common adverse events that can occur after injecting morphine (e.g., dizziness, drowsiness, sweating, low blood pressure and nausea; *see* Jamison et al., 1998). When the participant acknowledged the putative unpleasantness of receiving morphine, the study personnel told then participants that they were going to be injected with a dose of morphine of 3.5 mg which corresponded to half the dose that was routinely administered in a hospital setting. Moreover, he made sure to leave on the table a real morphine packaging in plain sight.

Then, after the injection of saline framed as morphine, the licensed personnel asked participants to remain seated and wait 15 min for morphine to reach its peak concentration. We carefully explained them that the choice of 15 min was grounded in the literature of morphine pharmacodynamics (*see for a review* Sverrisdóttir et al., 2015). During that time, we showed our participants a short documentary on anaesthesia in order to prime their verbally-induced expectations regarding morphine analgesic properties (https://youtu.be/UN4RNnIq7ds?t=699).

After 15 min, the licensed personnel assessed participants' cardiovascular function for the second time. We made sure that the readings displayed on the screen were lower than during the first measurement (e.g., HR = 88 beats/min; SBP = 119 mmHg; DBP = 65 mmHg; *taken from* Rouby et al., 1981). To do that, we preprogramed in advance a low measurement (performed on ourselves) that we again reloaded on the device's screen as soon as the second blood pressure measurement was done. Hence, the participants were not aware of their actual results and each of them saw the same blood pressure readings for the first and second measurements.

2.7 | Timeline

Estimation of the timeline for the completion of the study starting from the Stage 1 in-principle-acceptance (IPA) date can be found in Table 1. Stage 2 submission was estimated to occur at the end of February 2022.

2.8 | (Neuro-) physiological recordings and preprocessing

2.8.1 | Electroencephalography (EEG)

The EEG data was recorded at 1024 Hz using a 64 electrodes EEG Biosemi ActiveTwo® system referenced to

TABLE 1 Estimated timeline for the completion of the study since Stage 1 IPA

Months since IPA	+1	+2	+3	+4	+5	+6	+7	+8	+9	+10	+11	+12
Recruitment												
Testing												
Data Analysis												
Writing												
Stage 2 submission												

the common mode sense-driven right leg (CMS-DRL) ground (Biosemi, Amsterdam, Netherlands). Offline preprocessing and further statistical analyses were performed using custom MATLAB R2020b (MathWorks, Natick, Mass) scripts based on the EEGLab v2021.0 Toolbox (Delorme & Makeig, 2004) coupled with Cartool 3.91 (Brunet et al., 2011), Ragu (Koenig et al., 2011) and STEN 2.0 (developed by Jean-François Knebel and Michael Notter: https://doi.org/10.5281/zenodo.1164038).

2.8.2 | ERP preprocessing

We relied on a semi-automated MATLAB (MathWorks, Natick, Mass) toolbox, autoERP2.0 (Najberg & Wicht, 2021) relying on the EEGLab Toolbox (Delorme & Makeig, 2004). The pre-processing of the raw data was done accordingly:

- Re-referencing to Cz electrode and band-pass filtering (.5–40 Hz).
- Artifacts removal on continuous data with the EEGLab plugins, (i) CleanLine (sinusoidal, line noise frequencies removed: 50/100 Hz; *see* Mullen, 2012), (ii) Artifact Subspace Reconstruction (ASR: non-stationary signals > 10SD from mixing matrix calculated on a clean 'reference' section of the recording; *see* Mullen et al., 2015; Chang et al., 2018) and (iii) BLINKER with default settings (detection of eye blinks; *see* Kleifges et al., 2017).
- Epochs' segmentation time-locked to stimulus onset (100-ms pre- to 1000-ms post-stimulus onset; *see for similar parameters* Aslaksen et al., 2011).
- Baseline correction on the whole epoch window.
- Interpolation of bad channel(s) using multiquadric interpolation relying on radial basis functions (see Jäger, 2018; Jäger et al., 2016; Buhmann & Jäger, 2019). Electrodes were selected based on identification from the averaged ERPs.
- Epochs averaging for each participant and for the PreInject and PostInject phases separately.[5]
- Re-referencing to the common average reference.

[5]*We removed the step that excluded the four last trials of each block used for the VAS subjective pain ratings. Since we decreased the number of trials in each ERP from 48 to 24, rejecting four additional trials would result in a lower signal-to-noise ratio while these trials could safely be included in the ERP. The editorial approval for the deviation was obtained on 27 October 2021, after the commencement of data collection but prior to data analysis.*

2.8.3 | ERP analyses

After the ERP pre-processing, we determined the period of interest (POI) for the group-level analyses. The POI was defined based on the N2 and P2 ERP components on the group-averaged Global Field Power (GFP) waveform. GFP is a measure of the strength of electrical field potentials computed as the standard deviation of the mean voltage amplitude over all electrodes at a given time point (Michel & Murray, 2012). The GFP peak during the component-specific POI corresponds to the time point during each component with the highest signal-to-noise ratio (Michel & Murray, 2012). Each component was identified at the individual level based on the latency and topography of each GFP peak (i.e. GFP peak around 300 ms with vertex [fronto-central] negativity and 400 ms with vertex positivity, respectively for the N2 and P2 components; *see for reviews* Garcia-Larrea et al., 2003; Kakigi et al., 2004). Once the component was identified, the POI was determined as the component peak latency of the mean GFP ± 1SD of the individual GFP peaks. Then, the ERP was averaged for each participant separately over each component's POI. The scripts used to determine the POI are freely available following this address: https://github.com/CorentinWicht/GFPPeaks (Wicht, 2020). The resulting ERP topographic map were submitted to the GMD analysis. GMD indexes the differences in the underlying configuration of two distinct electric fields and is computed as the root mean square of the difference between the potentials measured at each electrode for the different experimental conditions normalized by instantaneous GFP (Brunet et al., 2011). Hence, GMD informs about distinct configuration of neural networks activated in each experimental condition. GMD was analysed with robust randomization statistics (*see* Habermann et al., 2018; Koenig et al., 2011) using 5,000 permutations per data point with an alpha threshold of $p < .05$ to estimate the significance of GMD differences.

The analysis of the GMD provides interpretative advantage over the analyses of local ERP waveforms since it takes into account the whole electrode montage and is reference-independent. Importantly, because GMD analyses are applied on strength-normalized electric field, we could rule out that any observed effect was confounded by quantitative variations in the response strength of the underlying generators.

2.8.4 | Cardiovascular readings

Cardiovascular readings (i.e., systolic (SBP) and DBP, HR) were performed (i) before the PreInject pain

induction phase and (ii) 15 min after the injection and before the PostInject pain induction phase (*see* Figure 2) to increase the credibility in our deceptive procedure (*see* Section 2.6.2), using an Omron M7 Intelli IT device (Omron Healthcare, Inc., Lake Forest, IL, USA).

2.9 | Data removal summary

We rejected trials if they met the following criteria:

2.9.1 | Behaviour (VAS)

- Intra-subject level behaviour:
  - To ensure a thorough filling of the VAS, trials with a RT shorter than 300 ms were excluded.
- Inter-subject level behaviour:
  - We used the median absolute deviation (MAD) to detect outliers participants (Leys et al., 2019), with default parameters (i.e. MAD range around the median of 1.4826).
  - After detecting outliers, if they were considered random (i.e. belonging to the distribution of interest) we left them in the dataset. If they were considered interesting outliers (i.e. influenced by an unknown moderator), we applied the Winsorization approach (Tukey & Mclaughlin, 1963; percentile observation $k = 5$) to avoid as much as possible rejecting data points that would have resulted in loss of power.

EEG:

- For ERPs, trials with (i) at least one time frame (TF) at one electrode with voltage ±80 μV (*see* Hartmann et al., 2016) or (ii) jump of more than 30 μV at one electrode from one TF to the next will be reject.

We removed the entire data of one participant if it met the following criteria:

- Behaviour[6]
  - Evidence of substantial computer errors (i.e., >80% of datapoints corrupted).
- EEG:
  - The minimum number of EEG trials for each task was lower than 16[7] trials for each to-be-averaged ERPs (*see* Boudewyn et al., 2018).

2.10 | Summary of statistical tests

The target alpha threshold for all statistical comparisons was set to 5% (see Table 2 for a list of contrasts).

For behavioural analyses, normality was assessed with the Shapiro–Wilks test together with the criteria of skewness and kurtosis within a ± 2 range for parametric analyses (Gravetter & Wallnau, 2013). In case of non-normality, we used equivalent non-parametric tests (i.e., Mann–Whitney U). Additionally, for all statistical tests, effect sizes were reported using Hedges' g_s *and* g_{av} (respectively for between- and within-subject designs, *see* Lakens, 2013).

For electrophysiological analyses (i.e., GMD), normality of data distribution was not assessed since we used non-parametric randomization statistics that are robust to asymmetrical distributions.

2.11 | Data availability

Coded study data, digital materials and analysis codes were uploaded to a public archive and can be downloaded at the following address: https://doi.org/10.5281/zenodo.6043795.

Additionally, the approved and published Stage 1 protocol can be downloaded at the following address: https://doi.org/10.5281/zenodo.4541048.

3 | RESULTS

Averages in the results section are reported as mean ± SD for parametric tests and as median/IQR for non-parametric tests.

3.1 | Study population

We recruited 75 participants, of whom (i) 2 were excluded at the beginning of the session based on inclusion/exclusion criteria and (ii) 3 were excluded from the analyses to comply with the quality checks (Figure 3). Our final sample was composed of 70 participants (65.7% females) aged 23.7 ± 5.2 years (see Figures S1 and S2).

[6] *In relation to the adjustments implemented in the Sanity Checks section (*i.e.*, further discussed in the emails exchange of the 19.11.2021), the second criterion was here removed. Since we are already controlling for the occurrence of the placebo effect in the sanity check, it was unnecessary to repeat it in this section.*

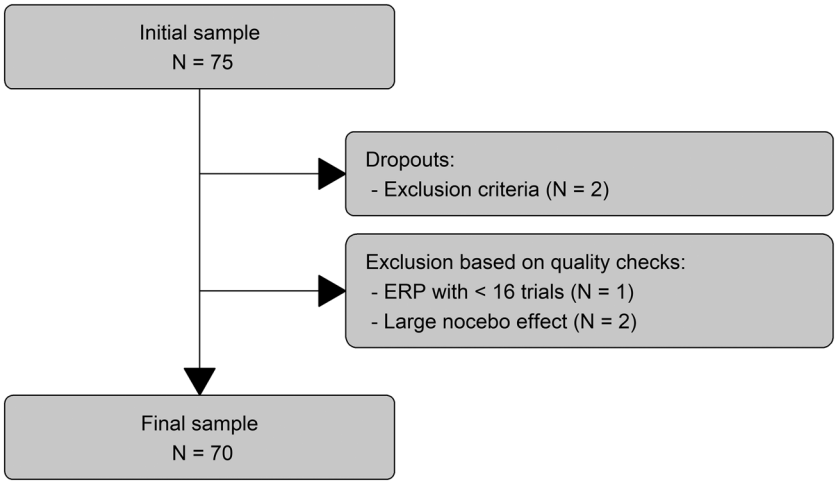
[7] *Since we decreased the number of trials in each ERP from 48 to 24 (*i.e.*, emails exchange of 27 October 2021), we adjusted this criterion.*

TABLE 2 Summary table of all statistical tests

Hypotheses	Statistical test	Contrast	Dependent variable	
			Behaviour	Physiology
Main contrast of interest				
PBO morphine effect	Independent-samples *t* test[a]	CondExp ≠[b] UncondExp		PostInject POI-averaged GMD values separately for: • N2 • P2

Note: GMD = Global Map Dissimilarity; PBO = Placebo; PostInject = Post-injection phase; VAS = Visual Analogue Score.
[a]One-sided test.
[b]The GMD index is not directional since it is a difference score between the two groups.

FIGURE 3 Study flow diagram



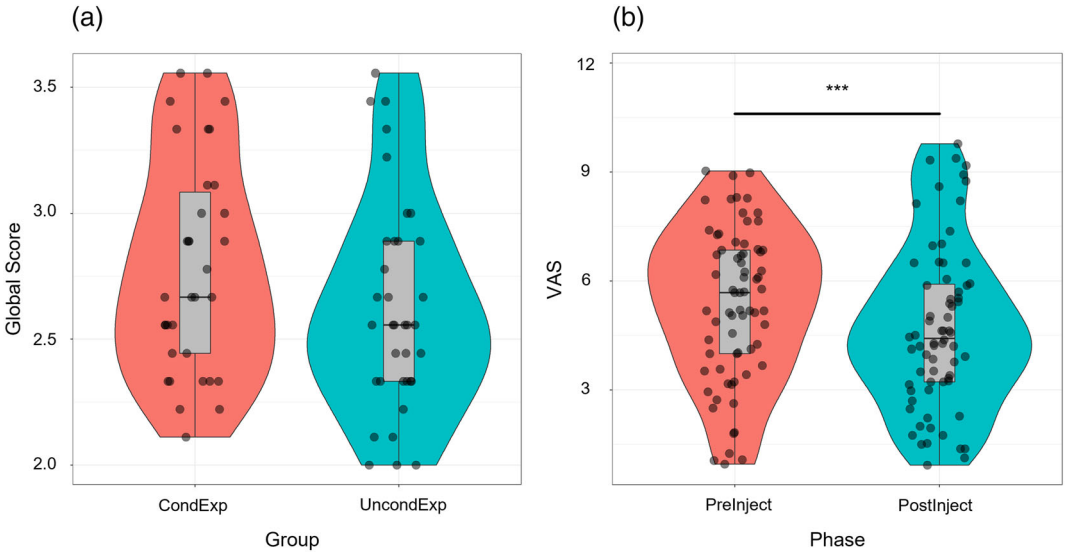


FIGURE 4 (a) Verbal expectations as measured with the morphine analgesia expectations questionnaire (MAExpQ) questionnaire averaged across groups. (b) Visual analogue scale (VAS) pain estimates averaged across phases. CondExp = Conditioned Expectations group; PreInject = pre-injection phase; PostInject = post-injection phase; UncondExp = Unconditioned Expectations group. *** = $p < .001$

The local ethics committee (*Commission cantonale d'éthique de la recherche sur l'être humain*, CER-VD) approved the protocol (#2021-00359). All recruited participants provided written informed consent prior to inclusion. They were all compensated for their participation.

3.2 | Sanity checks

Both sanity checks were validated (*see* Section 2.4.5): First, the groups difference in positive verbally-induced expectations of morphine analgesia was smaller than our criteria of $g_s < .4$ (actual difference of $g_s = .370$, Table S3 and Figure 4a). Second, we ensured that our intervention induced a PA of minimum $g_{av} \geq .5$ (actual PA of $g_{av} = .552$). To satisfy the second criteria, we had to replace four participants to ensure that a placebo effect indeed occurred after the injection (Table S4 and Figure 4b).

3.3 | Data removal

According to the rejection criteria in the Data Removal Summary section, no outliers were identified at the behavioural level. Regarding the EEG data, one participant (P1) was excluded for having <16 trials in one of the ERP. After its exclusion, we completed the sample so that each group included at least 33 participants, which corresponded to our minimum sample size (*see* Section 2.2.1).

TABLE 3 Group averages and SD of individual GFP peak, in milliseconds

Component	Group	Peak	SD
N2	UncondExp	456.86	40.01
	CondExp	450.47	51.66
	Overall	453.75	45.82
P2	UncondExp	589.45	50.42
	CondExp	585.06	43.30
	Overall	587.32	46.81

Note: GFP = Global Field Power; SD=Standard Deviation.

3.4 | ERPs results

As specified in the ERP analyses section, the Period of Interest (POI) for each of the investigated ERP component was determined as the average of individual GFP peaks ±1SD for each group (Table 3). While the N2 and P2 components were expected to occur respectively 200–400 and 300–500 ms post-stimulus onset (Bromm & Treede, 1987), they actually manifested in the 408- to 500- and 541- to 634-ms intervals (Figures S10 and S11).

3.5 | GMD

The GMD analyses did not confirm our hypothesis: we did not find evidence that participants with prior experience with opioids (i.e., CondExp group) recruit different brain networks during placebo analgesia compared to individuals without prior experience with opioids (i.e., UncondExp group) both during the N2 ($p = 0.951$, .61% explained variance, Figure 5a,b) and the P2 time windows, ($p = .605$, 1.07% explained variance, Figure 5c, d). Moreover, we computed the spatial correlation index to determine how strongly correlated the scalp topographies of the two groups were, separately for each component. As such, the two groups topographical maps were

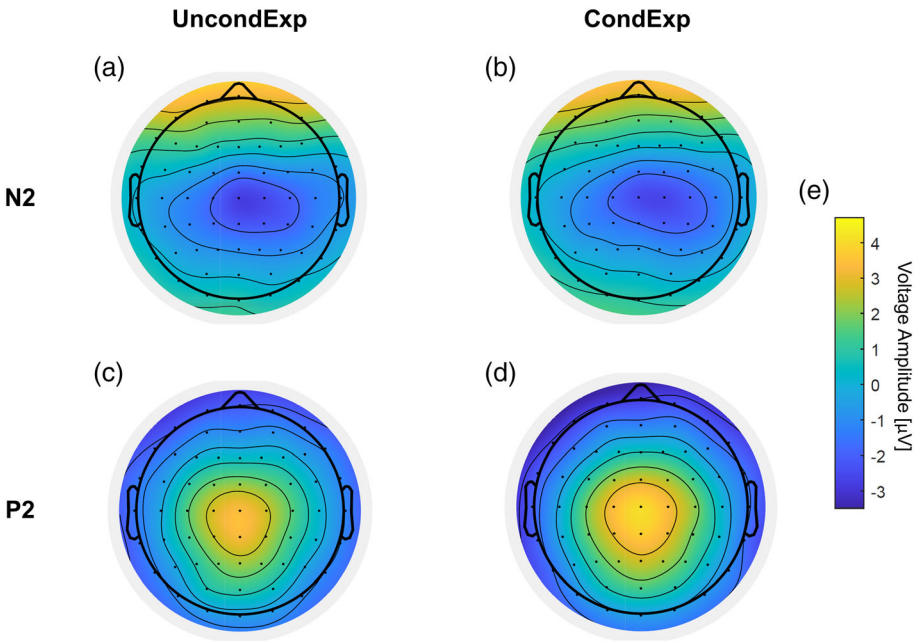


FIGURE 5 (a–d) topographical representations of voltage amplitudes (in µV) across groups (UncondExp and CondExp) and period of interest (POI) for each component of interests (N2 and P2). (e) Colour scale that was used to display the four topographies

strongly correlated both for the N2 ($r = .99$) and P2 ($r = .99$) components further supporting the assertion that the recruited brain networks were not different between groups.

3.6 | Exploratory findings

We conducted an exploratory GMD analysis using Ragu (Koenig et al., 2011) on the whole time-period to determine whether the ERP topographic group differences that we expected manifested outside our N2 and P2 POI or during short time periods.

We found two period of significant topographic modulations, namely 386 to 398 ms ($p = .035$, 3.02% explained variance) and 825 to 852 ms after the stimulus onset ($p = .022$, 3.5% explained variance; Figures 6a and 6c–g). The first significant period fell into the N2 time-window (Garcia-Larrea et al., 2003; Kakigi et al., 2004). The second significant period manifested during the

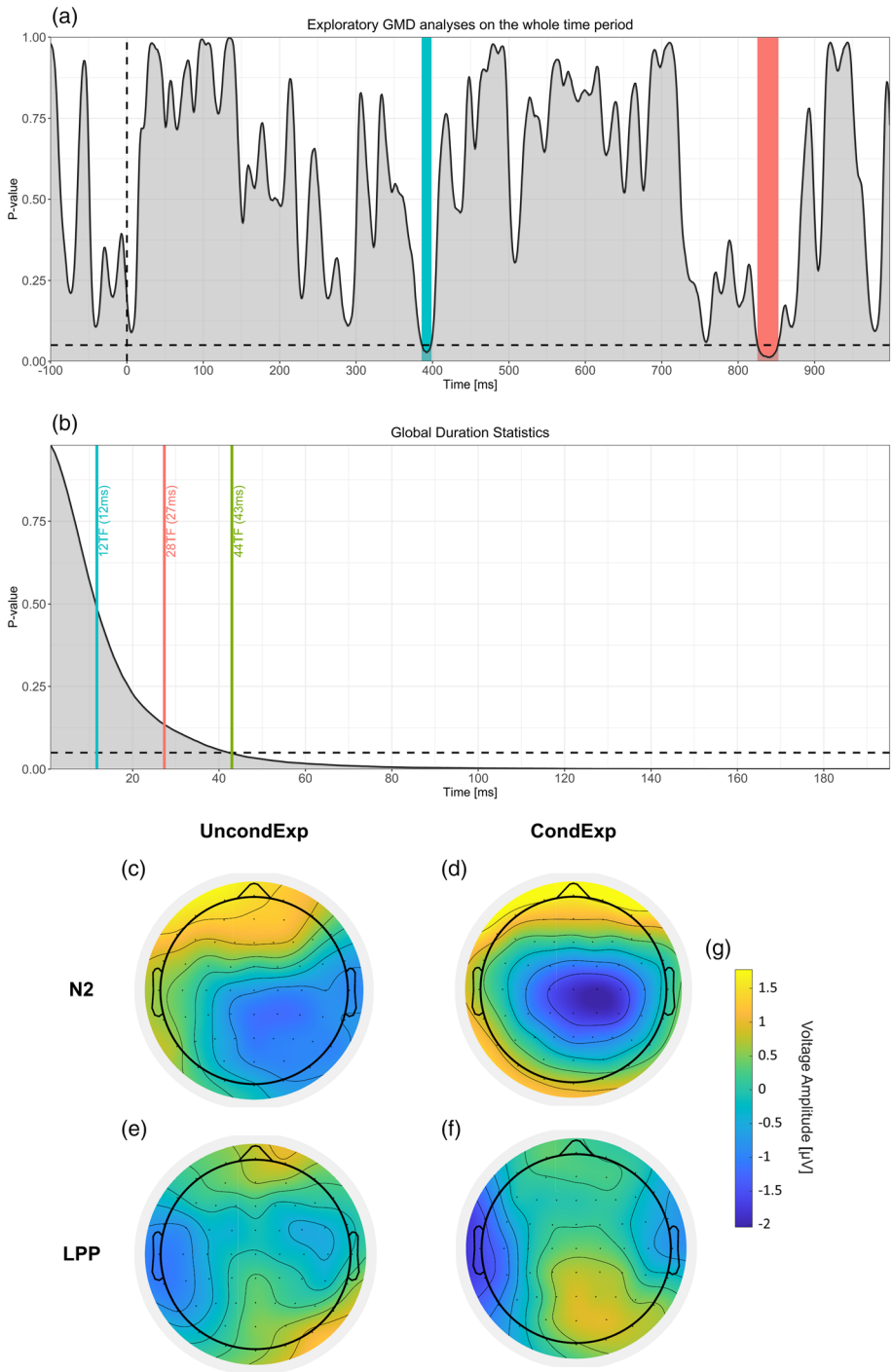


FIGURE 6 (a) *P* values of the Global Map Dissimilarity (GMD) differences between groups over time. The blue rectangle corresponds to the first significant time-period (N2) and the red one to the second (LPP). The dashed vertical line represents the stimulus onset. (b) *P* values of the global duration statistics over time. The vertical solid lines represent the length of each significant time-periods, namely 12 ms for N2 (blue), 27 ms for LPP (red) as well as the minimum duration needed to reach the 5% alpha level (43 ms in green). The *x* axis was shortened to improve visualization. (a,b) The dashed horizontal lines represent the 5% alpha level. (c–f) Topographical representations of voltage amplitudes (in µV) across groups (UncondExp and CondExp) and for the two significant time-periods (N2 and LPP). (g) Colour scale that was used to display the four topographies

centro-parietal late positive potential (LPP) component (usually 400- to 800-ms post-stimulus onset; Garland et al., 2015).

We additionally ran global duration statistics on the whole recording to identify the probability that for a N ms contiguous time-period of significant topographic modulation to occur under the null hypothesis (Figure 6b). We found that a minimum of 44 contiguous timeframes (i.e., ~43 ms) would have been required to reach a false positive rate of less than 5% (i.e., alpha level of $p < .05$). In our case, the first significant time-period lasted 12 ms while the second lasted 27 ms which corresponded to false positive rates of 48.59% and 13.48%, respectively. These short-lasting topographic modulations should thus be interpreted with much caution.

4 | DISCUSSION

The aim of the study was to test whether different types of morphine-related expectations would be supported by distinct brain networks during placebo analgesia (PA): We compared PA and the related electrophysiological activity between individuals with (CondExp group) vs without prior experience with morphine (UncondExp group) after being injected with a saline solution framed as being morphine. We posited that different brain networks would be recruited between the two groups over N2 and/or P2 ERP components after heat-pain stimulations. To test this hypothesis, we compared the ERP topography between the two groups. Topographic modulations indeed necessarily follow from alterations in the configuration of underlying brain generators (Murray et al., 2008; Tzovara et al., 2012).

Our positive control ensured that morphine analgesia was similar in the two groups ($g_s = .37$) and that PA indeed occurred following our sham morphine intervention ($g_{av} = .55$). In this context, however, our study could not contradict the assumption according to which ERP topographies are similar between the CondExp and UncondExp groups at the PostInject phase, neither during the N2 nor the P2 component. In the context of verbally-induced analgesia expectations, we did not find sufficient evidence to support the idea that prior conditioning to opioids leads to the recruitment of different brain networks to produce PA.

Assuming that our ERP analyses are sensitive to the differential recruitment of the dopaminergic and opioid systems, this finding is surprising since previous literature suggested that conditioned PA are supported by striatal regions of the dopaminergic reward system while the opioid system mainly underlie unconditioned PA (see for reviews de la Fuente-Fernández, 2009; Peciña et al., 2014; Okusogu & Colloca, 2019).

The absence of ERP topography effects on N2 and P2 components possibly originates from the fact that individuals in the CondExp group may have acquired expectations at first through verbal suggestions and then through conditioning, which may have led to a dominance of the former type of expectation in determining the underlying brain mechanisms. In a recent paper, Bajcar et al. (2021) indeed demonstrated that when verbal suggestions and classical conditioning coexist, the order of acquisition influences PA, with larger PA when verbal suggestions precede conditioning. Most importantly, Bajcar et al. (2021) have also found that when expectations were incongruent between verbal suggestions and conditioning, the magnitude of PA was in line with the direction of the last-used procedure. Since in clinical settings the experience of pain relief may precede suggestions provided by clinicians and follow from past positive and/or negative experiences, we cannot rule out that the order in which expectations were acquired, as well as their level of congruency, may have biased PA effects at neural level specifically in the CondExp group.

Additionally, whereas we formulated our hypothesis based on findings that conditioned and unconditioned expectations may recruit different neural networks to trigger PA (Carlino et al., 2015; Colloca et al., 2009), our study differs from this previous literature on several aspects. First, these studies focused on ERP components voltage amplitude of one electrode (Cz) that may reflect changes in ERP topography or changes in the global power of the ERP field. Since global field power modulations do not imply a modification in the configuration of the underlying neural generators (Murray et al., 2008; Tzovara et al., 2012), such metrics could not help testing our hypothesis and was thus ignored in the present study. In contrast, we focused on an index of topographic modulation insensitive to purely quantitative changes in field strength but able to detect modification of brain network configurations (Brunet et al., 2011). Second, the small sample sizes in previous studies (12–17 participants/group in Colloca et al., 2009; Carlino et al., 2015) may have resulted in overinflated effect size estimates and higher false positives/negatives rate (Button et al., 2013; Lakens, 2013; Schäfer & Schwarz, 2019). Since we estimated a sample size enabling to reach a 90% power to detect a medium effect size with a 5% α level, our results can be confidently interpreted as indicating that the effects of prior conditioning on the brain networks underlying placebo analgesia in the context of expectations is either

nonexistent or small. Of note, other neuroimaging techniques such as source estimations (Burle et al., 2015) or fMRI may have revealed brain network effects of prior conditioning that we could not detect with analyses of the field potential topography. Finally, the Colloca et al. (2009) and Carlino et al. (2015) experiments focused on laser-evoked potentials (LEPs), whereas we relied on contact heat evoked potentials (CHEPs). LEPs have been shown to be of shorter latency and of larger amplitude than CHEPs (De Schoenmacker et al., 2021). Together with the fact that each ERP only comprised on average 23.7 ± .8 trials, the use of CHEPs may have led to a signal-to-noise ratio too small to detect small topographic modulations. Further experiments relying on CHEPs may consider increasing the number of trials to compensate for smaller signal-to-noise ratio and reduced evoked amplitudes level.

5 | EXPLORATORY

We conducted exploratory ERP topography analyses over the whole ERP time-period to determine whether we may have failed to detect the true effect because of too large or inappropriate periods of interest. We observed two periods of significant topographic differences between groups, namely 386–398 and 825–852 ms post-stimulus onset. While the first period of significance falls right into the N2 component time-window described in the literature (Garcia-Larrea et al., 2003; Kakigi et al., 2004), it occurred slightly earlier than the POI we found (i.e., 408–500 ms; see Section 3.5). Regarding the second significant period, it is most likely related to the late segment of the late positive potential (LPP) component that Wang et al. (2020) hypothesized to index processes such as evaluation, memory and affect regulation (Zheng et al., 2019). Yet, based on the results of the global duration statistics, such short-lasting significant time-period (respectively 12 and 27 ms) should rather be considered as false positives than as potential indicators of true effects.

6 | CONCLUSIONS AND FUTURE DIRECTIONS

Our results do not confirm the hypothesis that different types of expectations depend on distinct PA-related brain networks. Future studies may control the order of acquisition and congruency of different types of expectations. Additionally, they may benefit from investigating the role that emotional arousal and associated brain areas play in mediating PA.

In sum, expectations formed through verbal suggestions or conditioning likely produce PA via corresponding brain network.

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CONFLICT OF INTEREST

All authors have declared no conflict of interest.

AUTHORS CONTRIBUTION

Wicht, Corentin Aurèle: Conceptualization, Methodology, Software, Formal Analysis, Investigation, Data Curation, Writing-Original Draft, Visualization, Funding Acquisition. **Mouthon Michael:** Data Collection, Methodology, Writing-Review & Editing. **Joelle Nsimire Chabwine**: Conceptualization, Methodology, Writing-Review & Editing. **Gaab, Jens**: Conceptualization, Methodology, Writing-Review & Editing. **Spierer, Lucas**: Conceptualization, Methodology, Resources, Writing-Review & Editing, Supervision, Project Administration, Funding Acquisition.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/ejn.15645.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available In Zenodo at https://doi.org/10.5281/zenodo.6043795.

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REFERENCES

Amanzio, M., & Benedetti, F. (1999). Neuropharmacological dissection of placebo analgesia: Expectation- activated opioid systems versus conditioning-activated specific subsystems. *The Journal of Neuroscience*, *19*, 484–494. https://doi.org/10.1523/JNEUROSCI.19-01-00484.1999

Apkarian, A. V., Bushnell, M. C., Treede, R. D., & Zubieta, J. K. (2005). Human brain mechanisms of pain perception and regulation in health and disease. *European Journal of Pain*, *9*, 463–484. https://doi.org/10.1016/j.ejpain.2004.11.001

Aslaksen, P. M., Bystad, M., Vambheim, S. M., & Flaten, M. A. (2011). Gender differences in placebo analgesia: Event-related

potentials and emotional modulation. *Psychosomatic Medicine*, *73*, 193–199. https://doi.org/10.1097/PSY.0b013e3182080d73

Bąbel, P. (2019). Classical conditioning as a distinct mechanism of placebo effects. *Frontiers in Psychiatry*, *10*, 1–6. https://doi.org/10.3389/fpsyt.2019.00449

Bajcar, E. A., Wiercioch-Kuzianik, K., Farley, D., Buglewicz, E., Paulewicz, B., & Bąbel, P. (2021). Order does matter: The combined effects of classical conditioning and verbal suggestions on placebo hypoalgesia and nocebo hyperalgesia. *Pain*, *162*, 2237–2245. https://doi.org/10.1097/j.pain.0000000000002211

Bartels, D. J. P., Van Laarhoven, A. I. M., Haverkamp, E. A., Wilder-Smith, O. H., Donders, A. R. T., Van Middendorp, H., Van De Kerkhof, P. C. M., & Evers, A. W. M. (2014). Role of conditioning and verbal suggestion in placebo and nocebo effects on itch. *PLoS ONE*, *9*(3), e91727. https://doi.org/10.1371/journal.pone.0091727

Benedetti, F. (2014). Placebo effects: From the neurobiological paradigm to translational implications. *Neuron*, *84*, 623–637. https://doi.org/10.1016/j.neuron.2014.10.023

Benedetti, F., Pollo, A., Lopiano, L., Lanotte, M., Vighetti, S., & Rainero, I. (2003). Conscious expectation and unconscious conditioning in analgesic, motor, and hormonal placebo/nocebo responses. *The Journal of Neuroscience*, *23*, 4315–4323. https://doi.org/10.1523/JNEUROSCI.23-10-04315.2003

Benedetti, F., Carlino, E., & Pollo, A. (2011). How placebos change the Patient's brain. *Neuropsychopharmacology*, *36*, 339–354. https://doi.org/10.1038/npp.2010.81

Boudewyn, M. A., Luck, S. J., Farrens, J. L., & Kappenman, E. S. (2018). How many trials does it take to get a significant ERP effect? It depends. *Psychophysiology*, *55*, 1–16. https://doi.org/10.1111/psyp.13049

Bromm, B., & Treede, R. D. (1987). Human cerebral potentials evoked by CO2 laser stimuli causing pain. *Experimental Brain Research*, *67*, 153–162. https://doi.org/10.1007/BF00269463

Brunet, D., Murray, M. M., & Michel, C. M. (2011). Spatiotemporal analysis of multichannel EEG: CARTOOL. *Computational Intelligence Neurosci.*, *2011*, 813870.

Buhmann, M., & Jäger, J. (2019). On radial basis functions. *Snapshots Mod. Math. From Oberwolfach*, *2*, 1–16.

Burle, B., Spieser, L., Roger, C., Casini, L., Hasbroucq, T., & Vidal, F. (2015). Spatial and temporal resolutions of EEG: Is it really black and white? A scalp current density view. *International Journal of Psychophysiology*, *97*, 210–220. https://doi.org/10.1016/j.ijpsycho.2015.05.004

Button, K. S., Ioannidis, J. P. A., Mokrysz, C., Nosek, B. A., Flint, J., Robinson, E. S. J., & Munafò, M. R. (2013). Power failure: Why small sample size undermines the reliability of neuroscience. *Nature Reviews. Neuroscience*, *14*, 365–376. https://doi.org/10.1038/nrn3475

Caldwell, A. & Lakens, D. (2019) Power analysis with superpower.

Carlino, E., Torta, D. M. E., Piedimonte, A., Frisaldi, E., Vighetti, S., & Benedetti, F. (2015). Role of explicit verbal information in conditioned analgesia. *Eur. J. Pain (United Kingdom)*, *19*, 546–553.

Chang, C.-Y., Hsu, S.-H., Pion-Tonachini, L., & Jung, T.-P. (2018). Evaluation of artifact subspace reconstruction for automatic EEG artifact removal. In *2018 40th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)* (pp. 1242–1245). https://doi.org/10.1109/EMBC.2018.8512547

Colloca, L. (2014). Placebo, nocebo, and learning mechanisms. *Handbook of Experimental Pharmacology*, *225*, 17–35. https://doi.org/10.1007/978-3-662-44519-8_2

Colloca, L., & Benedetti, F. (2009). Placebo analgesia induced by social observational learning. *Pain*, *144*, 28–34. https://doi.org/10.1016/j.pain.2009.01.033

Colloca, L., Benedetti, F., & Porro, C. A. (2008). Experimental designs and brain mapping approaches for studying the placebo analgesic effect. *European Journal of Applied Physiology*, *102*(4), 371–380. https://doi.org/10.1007/s00421-007-0593-6

Colloca, L., Tinazzi, M., Recchia, S., Le Pera, D., Fiaschi, A., Benedetti, F., & Valeriani, M. (2009). Learning potentiates neurophysiological and behavioral placebo analgesic responses. *Pain*, *139*, 306–314. https://doi.org/10.1016/j.pain.2008.04.021

De Jong, P. J., Van Baast, R., Arntz, A., & Merckelbach, H. (1996). The placebo effect in pain reduction: The influence of conditioning experiences and response expectancies. *International Journal of Behavioral Medicine*, *3*, 14–29. https://doi.org/10.1207/s15327558ijbm0301_2

de la Fuente-Fernández, R. (2009). The placebo-reward hypothesis: Dopamine and the placebo effect. *Park. Relat. Disord.*, *15*, 72–74.

De Pascalis, V., & Scacchia, P. (2019). The influence of reward sensitivity, heart rate dynamics and EEG-delta activity on placebo analgesia. *Behavioural Brain Research*, *359*, 320–332. https://doi.org/10.1016/j.bbr.2018.11.014

De Schoenmacker, I., Berry, C., Blouin, J. S., Rosner, J., Hubli, M., Jutzeler, C. R., & Kramer, J. L. K. (2021). An intensity matched comparison of laser- and contact heat evoked potentials. *Scientific Reports*, *11*, 1–12. https://doi.org/10.1038/s41598-021-85819-w

De Sola, H., Salazar, A., Dueñas, M., & Failde, I. (2018). Opioids in the treatment of pain. Beliefs, knowledge, and attitudes of the general Spanish population. Identification of subgroups through cluster analysis. *Journal of Pain and Symptom Management*, *55*, 1095–1104. https://doi.org/10.1016/j.jpainsymman.2017.12.474

Delorme, A., & Makeig, S. (2004). EEGLAB: An open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *Journal of Neuroscience Methods*, *134*, 9–21. https://doi.org/10.1016/j.jneumeth.2003.10.009

Elkins-Brown, N., Saunders, B., & Inzlicht, M. (2018). The misattribution of emotions and the error-related negativity: A registered report. *Cortex*, *109*, 124–140. https://doi.org/10.1016/j.cortex.2018.08.017

Fruhstorfer, H., Lindblom, U., & Schmidt, W. G. (1976). Method for quantitative estimation of thermal thresholds in patients. *Journal of Neurology, Neurosurgery, and Psychiatry*, *39*, 1071–1075. https://doi.org/10.1136/jnnp.39.11.1071

Garcí-Larrea, L., Peyron, R., Laurent, B., & Mauguière, F. (1997). Association and dissociation between laser-evoked potentials and pain perception. *NeuroReport*, *8*(17), 3785–3789. https://doi.org/10.1097/00001756-199712010-00026

Garcia-Larrea, L., Frot, M., & Valeriani, M. (2003). Brain generators of laser-evoked potentials: From dipoles to functional significance. *Neurophysiologie Clinique*, *33*, 279–292. https://doi.org/10.1016/j.neucli.2003.10.008

Garland, E. L., Froeliger, B., & Howard, M. O. (2015). Neurophysiological evidence for remediation of reward processing deficits in chronic pain and opioid misuse following treatment with mindfulness-oriented recovery enhancement: Exploratory ERP findings from a pilot RCT. *Journal of Behavioral Medicine*, *38*, 327–336. https://doi.org/10.1007/s10865-014-9607-0

Gravetter, F. J., & Wallnau, L. B. (2013). *Essentials of statistics for the behavioral sciences* (8th Ed. ed.). Cengage Learning.

Habermann, M., Weusmann, D., Stein, M., & Koenig, T. (2018). A student's guide to randomization statistics for multichannel event-related potentials using Ragu. *Frontiers in Neuroscience*, *12*, 1–20. https://doi.org/10.3389/fnins.2018.00355

Haour, F. (2005). Mechanisms of the placebo effect and of conditioning. *Neuroimmunomodulation*, *12*, 195–200. https://doi.org/10.1159/000085651

Hartmann, L., Sallard, E., & Spierer, L. (2016). Enhancing frontal top-down inhibitory control with go/NoGo training. *Brain Structure & Function*, *221*, 3835–3842. https://doi.org/10.1007/s00429-015-1131-7

Iannetti, G. D., Zambreanu, L., Cruccu, G., & Tracey, I. (2005). Operculoinsular cortex encodes pain intensity at the earliest stages of cortical processing as indicated by amplitude of laser-evoked potentials in humans. *Neuroscience*, *131*, 199–208. https://doi.org/10.1016/j.neuroscience.2004.10.035

Jäger, J. (2018) Advances in radial and spherical basis function interpolation.

Jäger, J., Klein, A., Buhmann, M., & Skrandies, W. (2016). Reconstruction of electroencephalographic data using radial basis functions. *Clinical Neurophysiology*, *127*, 1978–1983. https://doi.org/10.1016/j.clinph.2016.01.003

Jamison, R. N., Raymond, S. A., Slawsby, E. A., Nedeljkovic, S. S., & Katz, N. P. (1998). Opioid therapy for chronic noncancer back pain: A randomized prospective study. *Spine (Phila. Pa. 1976)*, *23*, 2591–2600. https://doi.org/10.1097/00007632-199812010-00014

Jin, J. F., Zhu, L. L., Chen, M., Xu, H. M., Wang, H. F., Feng, X. Q., Zhu, X. P., & Zhou, Q. (2015). The optimal choice of medication administration route regarding intravenous, intramuscular, and subcutaneous injection. *Patient Preference and Adherence*, *9*, 923–942. https://doi.org/10.2147/PPA.S87271

Kakigi, R., Inui, K., Tran, D. T., Qiu, Y., Wang, X., Watanabe, S., & Hoshiyama, M. (2004). Human brain processing and central mechanisms of pain as observed by electro- and magnetoencephalography. *J. Chinese Med. Assoc.*, *67*, 377–386.

Kirsch, I. (1997). Response expectancy theory and application: A decennial review. *Applied and Preventive Psychology*, *6*, 69–79. https://doi.org/10.1016/S0962-1849(05)80012-5

Kleifges, K., Bigdely-Shamlo, N., Kerick, S. E., & Robbins, K. A. (2017). BLINKER: Automated extraction of ocular indices from EEG enabling large-scale analysis. *Frontiers in Neuroscience*, *11*, 1–17. https://doi.org/10.3389/fnins.2017.00012

Koban, L., Brass, M., Lynn, M. T., & Pourtois, G. (2012). Placebo analgesia affects brain correlates of error processing. *PLoS ONE*, *7*, 1–8. https://doi.org/10.1371/journal.pone.0049784

Koenig, T., Kottlow, M., Stein, M., & Melie-García, L. (2011). Ragu: A free tool for the analysis of EEG and MEG event-related scalp field data using global randomization statistics. *Computational Intelligence and Neuroscience*, *2011*, 1–14. https://doi.org/10.1155/2011/938925

Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for *t*-tests and ANOVAs. *Frontiers in Psychology*, *4*, 1–12. https://doi.org/10.3389/fpsyg.2013.00863

Legrain, V., Guérit, J. M., Bruyer, R., & Plaghki, L. (2002). Attentional modulation of the nociceptive processing into the human brain: Selective spatial attention, probability of stimulus occurrence, and target detection effects on laser evoked potentials. *Pain*, *99*, 21–39. https://doi.org/10.1016/S0304-3959(02)00051-9

Legrain, V., Bruyer, R., Guérit, J. M., & Plaghki, L. (2003). Nociceptive processing in the human brain of infrequent task-relevant and task-irrelevant noxious stimuli. A study with event-related potentials evoked by CO 2 laser radiant heat stimuli. *Pain*, *103*, 237–248. https://doi.org/10.1016/S0304-3959(02)00451-7

Legrain, V., Bruyer, R., Guérit, J. M., & Plaghki, L. (2005). Involuntary orientation of attention to unattended deviant nociceptive stimuli is modulated by concomitant visual task difficulty. Evidence from laser evoked potentials. *Clinical Neurophysiology*, *116*, 2165–2174. https://doi.org/10.1016/j.clinph.2005.05.019

Leys, C., Delacre, M., Mora, Y. L., Lakens, D., & Ley, C. (2019). How to classify, detect, and manage univariate and multivariate outliers, with emphasis on pre-registration. *Int. Rev. Soc. Psychol.*, *32*, 1–10.

Mazoit, J. X., Butscher, K., & Samii, K. (2007). Morphine in postoperative patients: pharmacokinetics and pharmacodynamics of metabolites. *Anesthesia & Analgesia*, *105*(1), 70–78. https://doi.org/10.1213/01.ane.0000265557.73688.32

Meissner, K., Bingel, U., Colloca, L., Wager, T. D., Watson, A., & Flaten, M. A. (2011). The placebo effect: Advances from different methodological approaches. *Journal of Neuroscience*, *31*(45), 16117–16124. https://doi.org/10.1523/jneurosci.4099-11.2011

Michel, C. M., & Murray, M. M. (2012). Towards the utilization of EEG as a brain imaging tool. *NeuroImage*, *61*, 371–385. https://doi.org/10.1016/j.neuroimage.2011.12.039

Montgomery, G. H., & Kirsch, I. (1997). Classical conditioning and the placebo effect. *Pain*, *72*, 107–113. https://doi.org/10.1016/S0304-3959(97)00016-X

Mullen, T. (2012) CleanLine EEGLAB Plugin [WWW Document]. San Diego, CA Neuroimaging Informatics Tools Resour. Clear. House. URL https://www.nitrc.org/projects/cleanline

Mullen, T. R., Kothe, C. A. E., Chi, Y. M., Ojeda, A., Kerth, T., Makeig, S., Jung, T. P., & Cauwenberghs, G. (2015). Real-time neuroimaging and cognitive monitoring using wearable dry EEG. *IEEE Transactions on Biomedical Engineering*, *62*, 2553–2567. https://doi.org/10.1109/TBME.2015.2481482

Murray, M. M., Brunet, D., & Michel, C. M. (2008). Topographic ERP analyses: A step-by-step tutorial review. *Brain*

Topography, *20*, 249–264. https://doi.org/10.1007/s10548-008-0054-5

Muthén, L. K., & Muthén, B. O. (2002). How to use a Monte Carlo study to decide on sample size and determine power. *Structural Equation Modeling: A Multidisciplinary Journal*, *9*(4), 599–620. https://doi.org/10.1207/s15328007sem0904_8

Najberg, H. & Wicht, C.A. (2021) autoERP2.0.

Najberg, H., Wachtl, L., Anziano, M., Mouthon, M., & Spierer, L. (2020). Aging modulates prefrontal plasticity induced by executive control training. *Cereb. Cortex, under Revi.*, *31*, 809–825. https://doi.org/10.1093/cercor/bhaa259

Nakamura, Y., Donaldson, G. W., Kuhn, R., Bradshaw, D. H., Jacobson, R. C., & Chapman, C. R. (2012). Investigating dose-dependent effects of placebo analgesia: A psychophysiological approach. *Pain*, *153*, 227–237. https://doi.org/10.1016/j.pain.2011.10.024

National Health Institute. (2018) Morphine [WWW Document]. URL https://www.nhs.uk/medicines/morphine/

Okusogu, C., & Colloca, L. (2019). Placebo hypoalgesia: Above and beyond expectancy and conditioning. *Current Opinion in Behavioral Sciences*, *26*, 75–81. https://doi.org/10.1016/j.cobeha.2018.10.008

Olatunji, B. O., Ebesutani, C., Sawchuk, C. N., McKay, D., Lohr, J. M., & Kleinknecht, R. A. (2012). Development and initial validation of the medical fear survey-short version. *Assessment*, *19*, 318–336. https://doi.org/10.1177/1073191111415368

Pacheco-López, G., Engler, H., Niemi, M. B., & Schedlowski, M. (2006). Expectations and associations that heal: Immunomodulatory placebo effects and its neurobiology. *Brain, Behavior, and Immunity*, *20*, 430–446. https://doi.org/10.1016/j.bbi.2006.05.003

Paxton, P., Curran, P. J., Bollen, K. A., Kirby, J., & Chen, F. (2001). Monte Carlo experiments: Design and implementation. *Structural Equation Modeling: A Multidisciplinary Journal*, *8*(2), 287–312. https://doi.org/10.1207/s15328007sem0802_7

Peciña, M., Stohler, C. S., & Zubieta, J. K. (2014). Neurobiology of placebo effects: Expectations or learning? *Social Cognitive and Affective Neuroscience*, *9*, 1013–1021. https://doi.org/10.1093/scan/nst079

R Core Team. (2020) R: A language and environment for statistical computing.

Reicherts, P., Gerdes, A. B. M., Pauli, P., & Wieser, M. J. (2016). Psychological placebo and nocebo effects on pain rely on expectation and previous experience. *The Journal of Pain*, *17*, 203–214. https://doi.org/10.1016/j.jpain.2015.10.010

Ribordy Lambert, F., Wicht, C. A., Mouthon, M., & Spierer, L. (2020). Acute alcohol intoxication and expectations reshape the spatiotemporal functional architecture of executive control. *NeuroImage*, *215*, 116811. https://doi.org/10.1016/j.neuroimage.2020.116811

Rouby, J. J., Eurin, B., Glaser, O., Guillosson, J. J., Nafziger, J., Guesde, R., & Viars, P. (1981). Hemodynamic and metabolic effects of morphine in the critically ill. *Circulation*, *64*, 53–59. https://doi.org/10.1161/01.CIR.64.1.53

Schäfer, T., & Schwarz, M. A. (2019). The meaningfulness of effect sizes in psychological research: Differences between sub-disciplines and the impact of potential biases. *Frontiers in Psychology*, *10*, 1–13. https://doi.org/10.3389/fpsyg.2019.00813

Schenk, L. A., Sprenger, C., Geuter, S., & Büchel, C. (2014). Expectation requires treatment to boost pain relief: An fMRI study. *Pain*, *155*, 150–157. https://doi.org/10.1016/j.pain.2013.09.024

Stewart-Williams, S., & Podd, J. (2004). The placebo effect: Dissolving the expectancy versus conditioning debate. *Psychological Bulletin*, *130*, 324–340. https://doi.org/10.1037/0033-2909.130.2.324

Sverrisdóttir, E., Lund, T. M., Olesen, A. E., Drewes, A. M., Christrup, L. L., & Kreilgaard, M. (2015). A review of morphine and morphine-6-glucuronide's pharmacokinetic-pharmacodynamic relationships in experimental and clinical pain. *European Journal of Pharmaceutical Sciences*, *74*, 45–62. https://doi.org/10.1016/j.ejps.2015.03.020

Truini, A., Panuccio, G., Galeotti, F., Maluccio, M. R., Sartucci, F., Avoli, M., & Cruccu, G. (2010). Laser-evoked potentials as a tool for assessing the efficacy of antinociceptive drugs. *European Journal of Pain*, *14*, 222–225. https://doi.org/10.1016/j.ejpain.2009.05.001

Tukey, J. W., & Mclaughlin, D. H. (1963). Less vulnerable confidence and significance procedures for location based on a single sample: Trimming/Winsorization 1. *Sankhyā Indian J. Stat. Ser. A*, *25*, 331–352.

Tzovara, A., Murray, M. M., Michel, C. M., & De Lucia, M. (2012). A tutorial review of electrical neuroimaging from group-average to single-trial event-related potentials. *Developmental Neuropsychology*, *37*, 518–544. https://doi.org/10.1080/87565641.2011.636851

Voudouris, N. J., Peck, C. L., & Coleman, G. (1990). The role of conditioning and verbal expectancy in the placebo response. *Pain*, *43*, 121–128. https://doi.org/10.1016/0304-3959(90)90057-K

Wager, T. D., & Atlas, L. Y. (2015). The neuroscience of placebo effects: Connecting context, learning and health. *Nature Reviews. Neuroscience*, *16*, 403–418. https://doi.org/10.1038/nrn3976

Wager, T. D., Matre, D., & Casey, K. L. (2006). Placebo effects in laser-evoked pain potentials. *Brain, Behavior, and Immunity*, *20*, 219–230. https://doi.org/10.1016/j.bbi.2006.01.007

Walsh, D., Perin, M. L., & McIver, B. (2006). Pain from advanced Cancer: A prospective survey of intravenous. *American Journal of Hospice & Palliative Medicine*, *23*, 353–359. https://doi.org/10.1177/1049909106292170

Wang, K., Cai, G., Huang, S., Li, Y., Li, R., & Wu, W. (2020). Performance of healthy persons under pain in different cognitive load tasks: An event-related potential study on experimental pain individuals. *Brain and Behavior*, *10*(8). https://doi.org/10.1002/brb3.1713

Warbrick, T., Derbyshire, S. W. G., & Bagshaw, A. P. (2009). Optimizing the measurement of contact heat evoked potentials. *Journal of Clinical Neurophysiology*, *26*, 117–122. https://doi.org/10.1097/WNP.0b013e31819d8016

Wertli, M. M., Reich, O., Signorell, A., Burgstaller, J. M., Steurer, J., & Held, U. (2017). Changes over time in prescription practices of pain medications in Switzerland between 2006 and 2013: An analysis of insurance claims. *BMC Health Services Research*, *17*, 1–11. https://doi.org/10.1186/s12913-017-2086-6

Wicht, C.A. (2020) GFPPeaks.

Zheng, P., Lyu, Z., & Jackson, T. (2019). Effects of trait fear of pain on event-related potentials during word cue presentations that signal potential pain. *The European Journal of Neuroscience*, *50*, 3365–3379. https://doi.org/10.1111/ejn.14495

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